Participation of intracellular Ca$^{2+}$ stores in arteriolar conducted responses

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Yashiro, Yasuuki, and Brian R. Duling. Participation of intracellular Ca$^{2+}$ stores in arteriolar conducted responses. Am J Physiol Heart Circ Physiol 285: H65–H73, 2003. First published March 13, 2003; 10.1152/ajpheart.00662.2002.—We examined the role played by intracellular Ca$^{2+}$ stores in conducted vasomotor responses induced by phenylephrine (PE) in isolated hamster cremasteric arterioles. When applied briefly (~1 s) to isolated, cannulated arterioles by using pressure-pulse ejection from a micropipette, PE produced a strong local vasoconstriction and a very small biphasic conducted response (a small constriction followed by a dilation) that propagated several hundred micrometers along the vessel length. The conducted vasomotion was associated with a monophasic elevation of the endothelial cell intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) at the site of stimulation, as measured with the Ca$^{2+}$ indicator fura 2. The Ca$^{2+}$ pump inhibitor thapsigargin was used to limit filling of Ca$^{2+}$ stores in smooth muscle and endothelial cells. Thapsigargin reduced baseline diameter and elicited a strong dilator component at the local site while enhancing both the constriction and dilator components of the PE-induced conducted response. The enhanced conducted constractor component induced by thapsigargin was mimicked by extraluminal application of tetraethylammonium or charybdotoxin but not by iberiotoxin, apamin, glibenclamide, barium, or 4-aminopyridine. Thapsigargin increased the estimated basal endothelial cell [Ca$^{2+}$]$_i$ by ~60 nM and converted the PE-induced change in [Ca$^{2+}$]$_i$ from monotonic to biphasic with a late elevation of [Ca$^{2+}$]$_i$ above baseline that coincided with the increased dilatory component of the conducted response. Luminal application of charybdotoxin plus apamin significantly reduced the dilatory component of the conducted response. These results indicate that intracellular Ca$^{2+}$ stores play a dynamic role in regulating conducted vasomotor responses apparently through modulation of KCa channels in both cell types.

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Phenylephrine; K$^+$ channels; endothelium; intracellular Ca$^{2+}$ concentration

Longitudinal coordination of arteriolar tone is thought to be crucial for the precise regulation of the distribution of oxygen to tissues with changing metabolic needs (6, 13). Longitudinal conduction of vasomotor responses is induced by changes in membrane potential ($V_m$) that propagate along the vascular wall (36, 38, 39). The characteristics of the responses are dependent on a variety of factors and the agonist used. ACh evokes a conducted vasodilation via a hyperpolarization of the arterioles that is attributed to be either a production of endothelium-derived hyperpolarizing factor (EDHF) (37) or a direct conduction of hyperpolarizing current from endothelium to smooth muscle through myoendothelial gap junctions (9, 40).

Responses to constrictor stimuli are more complex. In the hamster cheek pouch in vivo, phenylephrine (PE) and norepinephrine (NE) commonly produce a rapid conducted constriction (2, 37), although arterioles from the hamster retractor in vivo show no conducted response to NE (31). Controversy exists regarding the ability of cremasteric arterioles to produce conducted response to PE in the mouse (14, 22), and in isolated arterioles from the hamster cremaster, PE causes a biphasic conducted response (small constriction followed by dilation, 41). Previously, we (41) have shown that the dilator component of the conducted response is endothelium dependent, as evidenced by the effects of intraluminal treatment with the Ca$^{2+}$ chelator BAPTA or the blockers of Ca$^{2+}$-dependent K$^+$ (KCa) channels charybdotoxin (CTX) and apamin. Because PE is a smooth muscle-specific agonist, we proposed that communication between the smooth muscle and endothelium plays a key role in the development of complex vasomotor responses, and we hypothesized that the communication is mediated by myoendothelial gap junctions.

Diversity in the PE response also arises from the fact that PE elicits vasoconstriction with two distinct mechanisms: electromechanical coupling and pharmacomechanical coupling (4, 33). Electromechanical coupling depends on smooth muscle depolarization, which promotes Ca$^{2+}$ entry through voltage-gated Ca$^{2+}$ channels. Elevated cytosolic Ca$^{2+}$ also modulates the subsequent gating of ion channels in the wake of depolarization (26). The membrane potential change associated with electromechanical coupling is also conducted down the length of the arteriole producing dis-
tant activation of voltage-sensitive Ca\textsuperscript{2+} channels and constriction.

Vasoconstriction resulting from pharmacomechanical coupling, on the other hand, is dependent on Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) and sensitization of the contractile proteins. In addition to raising global intracellular Ca\textsuperscript{2+}, SR activation releases Ca\textsuperscript{2+} into highly restricted subsarcolemmal space, producing “Ca\textsuperscript{2+} sparks” (17, 25), which may add to the contractile force either by contributing directly to the global elevation in cytoplasmic Ca\textsuperscript{2+} concentration or indirectly by increasing Ca\textsuperscript{2+} entry through depolarization caused by activation of Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (20, 27).

Ca\textsuperscript{2+} sparks may also exert a profound negative-feedback effect on vascular contraction via membrane hyperpolarization mediated by activation of large-conductance K\textsubscript{Ca} channels (17, 25). If K\textsubscript{Ca} channel activation also occurs during PE stimulation, then the initial depolarization would be reduced, leading to less electromechanical coupling and, at the same time, leading to reduced conducted vasoconstriction.

From these complex interactions among SR and ion channels, we hypothesized that the SR might play an important and complex role in determining the magnitude of PE-induced conducted vasmotor response. Furthermore, we hypothesized that K\textsubscript{Ca} channels would mediate the SR-related, conducted vasmotor responses. To test these hypotheses, we examined the effect of thapsigargin, a specific inhibitor of the Ca\textsuperscript{2+}-ATPase of intracellular stores, on vasmotor responses. We also tested the effects of several K\textsuperscript{+} channel blockers to ascertain the existence of a link between Ca\textsuperscript{2+} release and to K\textsuperscript{+} channel activation in the smooth muscle.

**MATERIALS AND METHODS**

*Isolated vessel preparation.* The University of Virginia Animal Care and Use Committee approved procedures and protocols used in this study. In all experiments, male Golden hamsters (130 ± 4 g, n = 30; Charles River Breeding Laboratories) were anesthetized with pentobarbital sodium (50 mg/kg ip), and the cremaster muscle was excised, freed of connective tissue, and spread out in a refrigerated (4°C) dissection chamber containing MOPS-buffered saline (in mM: 145 NaCl, 4.70 KCl, 2.0 CaCl\textsubscript{2}, 1.71 MgSO\textsubscript{4}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 5.0 glucose, and 2.0 MOPS) and 1% low endotoxin BSA (MOPS-BSA, United States Biochemical). Arterioles were isolated by blunt dissection, cannulated, and perfused according to methods described previously (5, 7). In brief, a segment of a first- or a second-order unbranched arteriole ~3 mm in length was dissected and transferred to a temperature-controlled tissue chamber, which was mounted on an inverted microscope (Olympus IMT-2). Arterioles were cannulated at both ends with glass pipettes attached to MOPS-BSA-filled reservoirs. The heights of the two reservoirs were initially set at a mean level of 40 mmHg (1 mmHg = 133 Pa) above the vessel with a gradient of 2.6 mmHg between the two ends. A 40- to 60-min equilibration period followed, during which the temperature in the bath was gradually raised to 37°C. Throughout the experiment, the tissue bath was flushed with MOPS-buffered saline at a rate of 2 ml/min, equivalent to a bath turnover rate of 1.5/min. For all agents, the appropriate vehicle controls were carried out and found to have no effect on vessel diameter or fluorescence responses. At the end of each experiment, maximal diameter was determined by exposure of the vessel to nominally Ca\textsuperscript{2+}-free MOPS-buffered saline with 0.1 mM adenosine at room temperature.

To stimulate the vessels, a pipette (tip diameter 5 μm) was positioned near the wall (<20 μm) with the aid of a motor-driven micromanipulator. All agonists were pressure ejected onto the abluminal surface of the vessel using a Pneumatic Pico Pump (PV 820, World Precision Instruments; Sarasota, FL), thereby allowing rapid and precise control of drug delivery. Small negative pressure applied to the stimulating pipette prevented leakage of the drug from the pipette tip. Both the superfusion and perfusion flows were directed along the length of the vessel toward a stimulating pipette placed at the distal end. This prevented diffusion or convection of the agonist from the site of stimulation to the site of observation. Under conditions used in these experiments, fluorescent dye (fluorescein, 332.31 mol wt) ejected from the stimulating pipette could be detected <200 μm in the upstream direction.

*Video microscopy.* Arterioles were visualized by transillumination at a magnification of ×900 (Olympus ×40, 0.7 numerical aperture objective). The image acquired by a video camera (Dage-MTI) was displayed on a video monitor (Olympus, OEV143) equipped with a video caliper (Microcirculation Research Institute, Texas A & M University) and recorded for subsequent off-line image analysis (model HR-S9500U, JVC). PicoPump and video-caliper voltages were sampled at 5 Hz by an analog-to-digital board and processed with MetaFluo system (Universal Imaging; West Chester, PA).

*Fluorescence imaging.* For measurement of endothelial cell intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}), the Ca\textsuperscript{2+}-sensitive indicator fura 2 was selectively loaded into endothelial cells by perfusing MOPS-BSA containing 5 μM fura 2-AM, 0.15% DMSO, and 0.015% Pluronic F127 for 5 min followed by a 20-min wash (10, 41).

Fluorescence images were visualized with epi-illumination (125 W xenon arc lamp) at a magnification of ×900 (Olympus ×40, 0.7 numerical aperture objective). Slits placed at an intermediate image plane limited the illuminated field to a 100-μm diameter, thereby reducing photic damage to the vessel. Fura 2 was excited at 340 and 380 nm, and the emission light was sampled at 510 nm with an intensified CCD camera (XR GenIII, Stanford Photonics; Stanford, CA). The images were recorded, digitized, and stored with the MetaFluo system (Universal Imaging) for subsequent [Ca\textsuperscript{2+}]\textsubscript{i} estimation from a region of interest. Endothelial [Ca\textsuperscript{2+}]\textsubscript{i} was estimated after subtracting background fluorescence, using the 340-to-380 ratio along with the in situ calibration parameters in the equation (12)

\[
[\text{Ca}^{2+}] = K_d[\text{R} - R_{\text{min}}]/(R_{\text{max}} - R)\beta
\]

where K\textsubscript{d} is the dissociation constant (224 nM); R is the 340 nm/380 nm ratio measurement; R\textsubscript{min} is the ratio in the presence of 0 Ca\textsuperscript{2+} MOPS plus 5 mM EGTA, 0.1 mM adenosine, and 10 μM ionomycin; R\textsubscript{max} is determined in the presence of 2.0 mM Ca\textsuperscript{2+} MOPS, 0.1 mM adenosine, and 10 μM ionomycin; and β is the ratio of fluorescence at 380 nm with 0 Ca\textsuperscript{2+} to 380 nm fluorescence with 1.2 mM Ca\textsuperscript{2+}. The Ca\textsuperscript{2+} calibration was performed on completion of each experiment.

**Protocols.** After the equilibration period, changes in arteriolar diameter in response to a short pulse of PE (1 mM pipette concentration, 0.5- to 1.0-s pulse) were observed at the site of the stimulating pipette (designated as “local” in the figures) and at sites 500 and 1,000 μm upstream from the arteriolar segment of a

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*Current and Ca\textsuperscript{2+} Signaling in Arterioles* • Vol 285 • July 2003 • www.ajpheart.org
stimulating pipette. This served as the control response. Arterioles were then perfused with thapsigargin (100 nM) for >20 min either from luminal or abluminal side, and the response to PE was reexamined. The concentration for thapsigargin was selected based on other studies (21, 25).

In preliminary experiments, we noted that prolonged incubation with thapsigargin or a frequent stimulation with agonists during thapsigargin treatment often caused a loss of tone. This appeared to be the result of a progressive accumulation of Ca^{2+}, and it could be prevented by coincubation with N^ω-nitro-l-arginine methyl ester (l-NAME, 10 μM), which was used in all experiments with thapsigargin. We have previously shown that l-NAME does not significantly alter the responses to short pulses of drug in this preparation (41).

After control data were obtained, vessels were superfused with a variety of K^+ channel blockers, and the response to PE was examined. The following blockers were used: 1) tetraethylammonium (TEA, 1 mM; nonspecific inhibitor of KCa channels); 2) iberiotoxin (IbTX, 100 nM) alone or in combination with apamin (500 nM) to block large- (BKCa) and small-conductance (SKCa) KCa channels, respectively; 3) charybdotoxin (CTX, 100 nM), a blocker of large and intermediate conductance KCa (IKCa) channels as well as voltage-sensitive K^+ (KV) channels; 4) glibenclamide (1 μM) to provide pharmacological block of ATP-sensitive K^+ (KATP) channels, 5) barium chloride (Ba^{2+}, 30 μM) to antagonize inward rectifier K^+ channels (IK); and 6) 4-aminopyridine (4-AP, 3 mM) to inhibit delayed rectifier K^+ channels (Kv). To discriminate the direct effect from the endothelium-dependent component, some of the K^+ channel blockers were applied intraluminally and the response to PE was compared.

**Chemicals.** Fura 2-AM and Pluronic F-127 were purchased from Molecular Probes (Eugene, OR). BSA was purchased from United States Biochemical (Cleveland, OH). l-NAME and CTX were purchased from RBI (Natick, MA). IbTX was purchased from Alomone Labs (Jerusalem, Israel). All other chemicals were purchased from Sigma Chemical (St. Louis, MO). TEA and barium chloride were dissolved in MOPS solution. IbTX and CTX were dissolved as 1 μM stock in MOPS solution with 0.1% BSA. Aliquots of the drugs were stored at −20°C until used. Apamin was reconstituted as 500 μM stock in 50 mM acetic acid. Glibenclamide was dissolved as 0.1 M stock in DMSO. 4-AP was dissolved in MOPS solution and adjusted to pH 7.4 with HCl.

**Statistical analysis.** Diameter changes were expressed as a percentage of the baseline diameter at the onset of PE stimulation. Zero time in all cases is the time at which drug application commenced. Data are expressed as means ± SE. The n value represents the number of vessels used. Differences within and between groups were compared using ANOVA, followed by Bonferroni or Fisher’s protected least significant difference multiple-range test. A value of P < 0.05 was considered significant.

**RESULTS**

**Effect of thapsigargin on local and conducted PE responses.** During the equilibration period, arterioles developed tone, contracting to roughly 50% of their maximal diameter (106 ± 2 to 52 ± 2 μm, n = 41). Typical responses of an isolated arteriole to a PE pulse before and during thapsigargin treatment are shown in Fig. 1A, and summary data are shown in Fig. 1B. In control conditions, PE caused a strong vasoconstriction at the site of stimulation and a very small, biphasic conducted response (constriction followed by dilation) at two upstream sites. Treatment with thapsigargin reduced baseline diameter by ~20% (52 ± 7 vs. 39 ± 5 μm, P < 0.05), and during the thapsigargin treatment, PE caused a vasoconstriction at the site of stimulation, but the onset of vasoconstriction was slower than in control conditions. The local vasoconstriction was followed by a significant dilation and the diameter returned to the initial level within 1 min (Fig. 1B, left).

![Fig. 1. A: responses to a brief pulse (1.0 s) of phenylephrine (PE, 1 mM) at local and two upstream sites (500 and 1,000 μm) before and during application of thapsigargin (100 nM). A: representative traces of changes in vessel diameter before (solid line) and during (dashed line) thapsigargin treatment. B: summarized data of the PE-induced responses (n = 8). Diameters are expressed as a percentage of the baseline diameter at the onset of PE stimulation. Values are means ± SE. *P < 0.05 vs. control.](http://ajpheart.physiology.org/).
Thapsigargin was tested in both the bath and the vessel perfusate. The vascular effects of thapsigargin were identical with the two modes of application, as would be predicted from the high-lipid solubility of the drug. Therefore, results obtained with the two modes of application were pooled. At the upstream sites where conducted vasomotor responses occurred, both constrictor and dilator component of the PE-induced conducted response was significantly enhanced by thapsigargin (Fig. 2).

Vessels were also stimulated with a short pulse of KCl (250 mM, 2.5 s) before and during the thapsigargin treatment (Fig. 3). In control conditions, KCl caused a rapid local vasoconstriction, presumably by electromechanical coupling to L-type Ca\(^{2+}\) channels. The conducted constriction was often followed by a very small vasodilation. As shown in Fig. 3, thapsigargin treatment did not augment the KCl-induced local or conducted vasoconstrictions. At the site of stimulation, however, KCl-induced vasoconstriction was followed by a quick diameter recovery with a small vasodilation. At the upstream sites, a larger conducted vasodilation was signiﬁcantly enhanced by thapsigargin (Fig. 2).

Effect of extraluminally applied K\(^{+}\) channel blockers on the PE-induced responses. To test for a possible link between intracellular Ca\(^{2+}\) release and K\(_{\text{Ca}}\) channel activation in smooth muscle, the PE-induced conducted response was tested before and during extraluminal application of K\(_{\text{Ca}}\) channel blockers (Fig. 4). CTX reduced the baseline diameter by \(-10\%\) (58 ± 4 vs. 52 ± 4 μm, \(P < 0.05\)) and a small oscillatory vasomotion (7–10 cycles/min, 3–5 μm amplitude). At the local site, CTX extended the duration of the PE-induced vasoconstriction (\(P < 0.05\)), and at upstream sites, it eliminated the dilator component and enhanced both the magnitude and duration of the PE-induced conducted constriction. Table 1 summarizes effects of all the K\(^{+}\) channel blockers tested on the PE-induced conducted responses. Administration of TEA caused a significant reduction of baseline diameter by \(-20\%\) (50 ± 3 vs. 40 ± 3 μm, \(P < 0.05\)) with a small spontaneous oscillation (6–10 cycles/min, 5–7 μm amplitude). TEA sharply enhanced the constrictor component of the PE-induced conducted response but did not alter the dilatory component. IbTX neither reduced the baseline diameter (54 ± 3 vs. 52 ± 4 μm, \(P > 0.05\)) nor produced vasomotion. IbTX did enhance the duration of the PE-induced vasoconstriction at the stimulated site but had negligible effect on the conducted responses. Addition of apamin to the IbTX-treated vessels also produced no effect on both the baseline diameter and the PE-induced responses. Glimepiride, barium, and 4-AP all produced significant reductions of the baseline diameter. Of the three 4-AP-treated vessels, one elicited a small vasomotion superimposed on the increased tone. None of these treatments, however, elicited significant changes in the magnitude of PE-induced conducted response.

CTX and TEA were also applied from the intraluminal side to test whether extraluminal application of the toxins is more specific to the smooth muscle. Intraluminal application of CTX or TEA neither reduced the baseline diameter nor augmented the PE-induced conducted response. The dilator component was reduced slightly but signiﬁcantly by CTX but not by TEA. These data are also summarized in Table 1.

Changes in endothelial cell [Ca\(^{2+}\)]\(_{\text{i}}\), before and during thapsigargin treatment. To explore the potential for an endothelial cell contribution to the vasomotor responses, changes in endothelial cell [Ca\(^{2+}\)]\(_{\text{i}}\) in response to PE were measured and these are summarized in Fig. 5. In control conditions, PE caused a significant rise in endothelial cell [Ca\(^{2+}\)]\(_{\text{i}}\). Thapsigargin increased base-
line Ca\(^{2+}\) level by \(-60\) nM (114 ± 5 vs. 180 ± 19 nM, \(P < 0.05\)). In the presence of thapsigargin, the initial rise in [Ca\(^{2+}\)], produced by PE, was followed by a rapid reduction of Ca\(^{2+}\), to levels well below baseline, and finally to levels above baseline. This biphasic response was associated with a comparable pattern of conducted vasomotion.

To further test the involvement of an endothelial component in the vasomotor response, vessels were perfused intraluminally with a combination of the KC\(_a\) channel blockers, CTX and apamin, during thapsigargin treatment. Typical responses are depicted in Fig. 6A, and summarized data are shown in Fig. 6B. We have previously shown that this combination was effective in eliminating dilatory component of the PE-induced conducted response in control conditions (41). As shown in Fig. 6, the combination was also effective in eliminating the enhanced PE-induced conducted vasodilation seen in the presence of thapsigargin.

**DISCUSSION**

The major findings of the present study are that: 1) blockade of intracellular Ca\(^{2+}\) stores with thapsigargin reduces baseline arteriolar diameter and augments both the constrictor and the dilator component of the conducted vasomotor response to PE; 2) extraluminal application of blockers of calcium-sensitive K\(^+\) channels mimics the enhanced constrictor component of the conducted response, but application of blockers of K\(_{ATP}\), K\(_{ir}\), or K\(_V\) channels do not; 3) the enhanced dilator component of the PE-induced conducted response is associated with a secondary, local increase of endothelial cell [Ca\(^{2+}\)], and intraluminal application of KC\(_a\) channel blockers inhibits the conducted dilation. Thus we conclude that intracellular Ca\(^{2+}\) stores play a complex and dynamic role in regulating the magnitude of the PE-induced conducted responses, apparently by the mediation of a change in the conductance of one or more K\(^+\) channels, one of which is located in the endothelium.

**Increased constrictor component of the PE-induced conducted response.** One might speculate that the increased conducted constriction following thapsigargin treatment was the result of a reduced Ca\(^{2+}\)-buffering capacity as a result of SR inhibition. If that were the case, KCl-induced conducted constriction should also have been modulated by thapsigargin. As shown in Fig. 3, however, thapsigargin had a negligible effect on the magnitude of KCl-induced conducted constriction. Thus one must look for other mechanisms.

**Table 1. Effects of various K\(^+\) channel blockers on PE-induced conducted response obtained 500 \(\mu\)m upstream from the stimulated site**

<table>
<thead>
<tr>
<th>(K^+) Channel Blockers</th>
<th>(n)</th>
<th>Change in Baseline Diameter, %</th>
<th>Max. Constriction, (\mu)m</th>
<th>Max. Dilation, (\mu)m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraluminal application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX</td>
<td>5</td>
<td>(-10 \pm 3^*)</td>
<td>(14 \pm 2^†)</td>
<td>(11 \pm 1)</td>
</tr>
<tr>
<td>TEA</td>
<td>7</td>
<td>(-19 \pm 6^†)</td>
<td>(13 \pm 2^†)</td>
<td>(8 \pm 2)</td>
</tr>
<tr>
<td>IbTX</td>
<td>5</td>
<td>(-3 \pm 5)</td>
<td>(3 \pm 1)</td>
<td>(11 \pm 2)</td>
</tr>
<tr>
<td>IbTX + apamin</td>
<td>5</td>
<td>(1 \pm 3)</td>
<td>(3 \pm 0)</td>
<td>(11 \pm 2)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>4</td>
<td>(-9 \pm 3^*)</td>
<td>(3 \pm 0)</td>
<td>(12 \pm 2)</td>
</tr>
<tr>
<td>Ba(^{2+})</td>
<td>4</td>
<td>(-10 \pm 1^*)</td>
<td>(3 \pm 1)</td>
<td>(12 \pm 3)</td>
</tr>
<tr>
<td>4-AP</td>
<td>4</td>
<td>(-26 \pm 5^†)</td>
<td>(4 \pm 1)</td>
<td>(9 \pm 1)</td>
</tr>
<tr>
<td>Intraluminal application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX</td>
<td>5</td>
<td>(-2 \pm 1)</td>
<td>(3 \pm 1)</td>
<td>(4 \pm 2^*)</td>
</tr>
<tr>
<td>TEA</td>
<td>3</td>
<td>(0 \pm 3)</td>
<td>(3 \pm 2)</td>
<td>(11 \pm 4)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Magnitudes of the phenylephrine (PE)-induced conducted responses before (control) and during application of charybdotoxin (CTX, 100 nM), tetraethylammonium (TEA, 1 mM), iberiotoxin (IbTX, 100 nM), IbTX (100 nM) plus apamin (500 nM), glibenclamide (1 \(\mu\)M), barium chloride (Ba\(^{2+}\), 30 \(\mu\)M) or 4-aminopyridine (4-AP, 3 mM) were compared. *\(P < 0.05\) vs. control. †\(P < 0.01\) vs. control.
From prior studies that have shown that the conducted response is the result of conduction of membrane potential change along the vessel axis (2, 31, 36, 38), the enhanced conducted constriction is most likely a reflection of a greater depolarization at the local site. A greater depolarization at the local site is the predicted outcome of a reduction in competing hyperpolarizing influences. It has been shown previously that activation of smooth muscle with PE causes a secondary increase in endothelial \([Ca^{2+}]_i\), via movement of Ca\(^{2+}\) or inositol 1,4,5-trisphosphate through myoendothelial gap junctions (5). A rise in endothelial cell Ca\(^{2+}\) triggers production of hyperpolarizing signals that are coupled via myoendothelial cell connections to smooth muscle hyperpolarizing signals and reduced Ca\(^{2+}\) influx. Buffering of endothelial cell Ca\(^{2+}\) or blockade of KCa channels eliminated the dilator component of the conducted response and converted it to a slowly developing, prolonged constriction (41). In isolated arterioles, the development of the conducted constriction, however, was fairly slow and was far from the rapid conducted constriction seen in the hamster cheek pouch in vivo or with KCl stimulation (2, 36).

The present experiments emphasize the importance of understanding the relative contributions of pharmacomechanical and electromechanical coupling to a vasomotor response. Only the latter directly produces a signal that is conducted (36, 38), and the response to PE involves both pharmacomechanical and electromechanical stimuli that sum in complex ways. Apparently, inhibition of uptake into intracellular Ca\(^{2+}\) stores exaggerated both constrictor and dilator components of the responses to PE (Figs. 1 and 2). The effect of the SR uptake blockade appears to be due both to: 1) reduced Ca\(^{2+}\) release with PE stimulation, and 2) to the secondary effects of Ca\(^{2+}\) on electromechanical coupling.

Fig. 5. Estimated global changes in endothelial cell intracellular Ca\(^{2+}\) concentration \([Ca^{2+}]_i\) in response to an abluminal pressure-pulse application of PE (1 mM, 1.0 s pulse) at the site of stimulation before and during thapsigargin treatment \((n = 4)\). Values are means \pm SE. *P < 0.05 vs. control. Significant changes \((P < 0.05)\) in \([Ca^{2+}]_i\) during the thapsigargin treatment from values at 0 s.

Fig. 6. Effect of intraluminal perfusion of CTX (100 nM) plus apamin (500 nM) on the PE-induced local and conducted responses during thapsigargin treatment. A: typical responses. B: summarized data \((n = 4)\). Vasodilations were significantly reduced at both local and upstream sites by luminal administration of the KCa channel blockers. Values are means \pm SE. *P < 0.05 vs. control.
Enhanced conducted dilation induced by thapsigargin. Inhibition of uptake into Ca$^{2+}$ stores increased not only the constrictor component, but also the dilator component of the PE-induced conducted response (Figs. 1 and 2). Previously, we showed that in control conditions, the dilatary component of the PE-induced conducted response was endothelium dependent (41). Two observations support the idea that the increased local contractions, as well as the conducted dilation observed during thapsigargin treatment, are likely to be endothelium dependent. First, both are associated with changes in endothelial cell [Ca$^{2+}$]i at the site of stimulation (Fig. 5). Second, intraluminal application of CTX and apamin significantly reduced the dilation (Fig. 6B).

In resting conditions, thapsigargin increased basal endothelial [Ca$^{2+}$]i, a finding consistent with previous reports showing that blockade of uptake leads to increased Ca$^{2+}$ levels (28, 32, 35). In the presence of thapsigargin, PE stimulation caused a greater rise of endothelial cell [Ca$^{2+}$]i (Fig. 5). Because PE acts primarily on the smooth muscle, the increase in endothelial [Ca$^{2+}$]i might represent Ca$^{2+}$ movement from smooth muscle to endothelium through myoendothelial gap junctions. After thapsigargin treatment, the initial endothelial cell Ca$^{2+}$ elevation was followed by a secondary decrease and a late increase above the baseline. The reasons for such dynamic changes in endothelial cell Ca$^{2+}$ are unknown. The secondary decrease might represent Ca$^{2+}$ movement between the two cell types that can be observed in the reduced Ca$^{2+}$-buffering state caused by diminished SR Ca$^{2+}$-buffering capacity. Alternatively, it may represent Ca$^{2+}$ efflux driven by the change in electrochemical driving force produced by the depolarization (30). We infer that that the delayed Ca$^{2+}$ elevation was associated with hyperpolarizations since local and conducted dilator responses were noted.

The hyperpolarization might have been induced by a production of EDHF or by a direct activation of K$\text{Ca}$ channels in the endothelium triggered by the initial rise in endothelial [Ca$^{2+}$]i. We note that the hyperpolarization itself might further promote Ca$^{2+}$ influx (30). In addition, reduced Ca$^{2+}$-buffering capacity of endothelium by the store inhibition might have lengthened the period of elevated [Ca$^{2+}$]i.

The dilatory component of the PE-induced conducted response during the thapsigargin treatment was eliminated by simultaneous administration of CTX and apamin (Fig. 6A). We (41) have previously shown that simultaneous administration of CTX and apamin, but not IbTX and apamin, in the lumen eliminates the dilator component of the PE-induced conducted response (in the absence of thapsigargin). We also demonstrated in the present study that intraluminal CTX does not change the baseline diameter and partially attenuates the dilator component of the PE-induced conducted response (Table 1). These findings may indicate that both IK$\text{Ca}$ and SK$\text{Ca}$ channels in the endothelium are involved in the spreading dilation as in the case of EDHF-induced vasodilation in rat hepatic artery (1). Alternatively, apamin may serve to help CTX binding to the IK$\text{Ca}$ channel (42). The fact that intraluminal TEA was ineffective in blocking the spreading dilation (Table 1) might reflect lower affinity for TEA for the IK$\text{Ca}$ channels in the endothelium (29). The endothelial hyperpolarization is expected to transmit to the smooth muscle via myoendothelial gap junctions, thereby causing vasodilation, although involvement of a diffusible factor such as epoxyeicosatrienoic acid or K$^+$ cannot be ruled out (3, 8).

Thapsigargin-induced vasoconstriction at rest. In resting conditions, thapsigargin caused a significant reduction of the arteriolar diameter. If SR Ca$^{2+}$ release is directly linked to the activation of K$\text{Ca}$ channels that are sensitive to TEA or CTX, then the constriction is likely to be, at least in part, due to inactivation of K$\text{Ca}$ channels. Although TEA was able to induce vasoconstriction of a similar magnitude (see RESULTS), inhibition of K$\text{Ca}$ channels is unlikely to be the only mechanism responsible for the enhanced vascular tone. In addition to the effect on K$\text{Ca}$ channels, depletion of intracellular Ca$^{2+}$ stores might lead to an increased capacitance Ca$^{2+}$ influx (23). SR blockade can also lead to reduction of Ca$^{2+}$-buffering capacity of the smooth muscle and contribute to the increase in [Ca$^{2+}$]i (34). Indeed, superfusion of TEA in the presence of thapsigargin caused further vasoconstriction and vasomotion (data not shown). The data also indicate that SR Ca$^{2+}$ release is not the only stimulant of K$\text{Ca}$ channels in resting conditions. We believe, however, that SR is an efficient source of Ca$^{2+}$ leading to K$\text{Ca}$ channel activation during the PE-induced vasoconstriction.

Coupling of SR Ca$^{2+}$ release to membrane potential changes. A K$\text{Ca}$ channel appears to play an additional role in attenuating the constrictor response. Because TEA or CTX applied from extraluminal side also augmented PE-induced conducted constrictions, we propose that SR Ca$^{2+}$ release is linked to activation of a K$\text{Ca}$ channel in the smooth muscle. The channel activated is unlikely to be the classic BK$\text{Ca}$ channel because IbTX, a highly specific blocker of BK$\text{Ca}$ channels, failed to enhance the PE-induced conducted constrictions. IbTX also had a negligible effect on baseline arteriolar diameter but was able to prolong local vasoconstriction in response to PE (data not shown). These data are compatible with previous findings by Jackson and Blair (16) that BK$\text{Ca}$ channels are silent in cremasteric arterioles at rest but that can be recruited during agonist-induced vasoconstriction. TEA is known as a nonspecific blocker of K$\text{Ca}$ channels but is also reported to inhibit K$\text{ATP}$ channels (19, 24). In addition to its inhibitory effect on BK$\text{Ca}$, CTX is a blocker of other channels including IK$\text{Ca}$ (11, 15) and Kv channels in some preparations (18). In the present study, neither apamin (in combination with IbTX), glibenclamide, nor 4-AP was effective in producing rapid conducted constriction. Taken together, these data support the idea that the smooth muscle IK$\text{Ca}$ channels are the most likely candidates to have been activated by SR Ca$^{2+}$ release during the PE stimulation. TEA caused a transient increase in PE-induced conducted constriction,
whereas CTX induced a prolonged constriction in addition to the rapid constrictor component (Fig. 4, Table 1). Such differences may be related to the fact that TEA, when applied intraluminally, does not block the dilatory component of the PE-induced conducted response, whereas luminal CTX partially attenuates the dilation (Table 1).

Concluding remarks. Our study shows that PE induces conducted responses, though they are small. Because no conducted vasomotor responses to PE was found in the mouse, it has been proposed that PE need not cause conducted constriction in some vascular beds because perivascular nerve plexuses can play the necessary role (14, 31). However, our data indicate that very complex signaling at the site of application of a drug can initiate many competing responses. Thus small shifts in the dominance of one or the other can produce significant shifts in the overall response pattern.

It appears that intracellular Ca\(^{2+}\) stores are an important regulator that determines the magnitude and/or duration of the agonist-induced conducted response in hamster cremasteric arterioles in vitro. Ca\(^{2+}\) stores seem to buffer changes in \(V_m\) through a secondary modulation of K\(_{Ca}\) channels in both cell types. These interactions help to reconcile conflicting reports on the efficacy of PE in inducing local and conducted vasomotor responses in the mouse (14, 22). The data shown in Figs. 1B, 2, and 3 show that conducted responses are likely to be the summation of dilator and constrictor components that can easily offset one another. Any situation that shifts the balance between pharmacomechanical and electromechanical will influence the magnitude of the conducted response. Further investigation will be required regarding the regulatory mechanism of the agonist-induced conducted responses in arterioles to elucidate their physiological significance.

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